

V483a – an Emerging Mutation Hotspot of Sars-Cov-2

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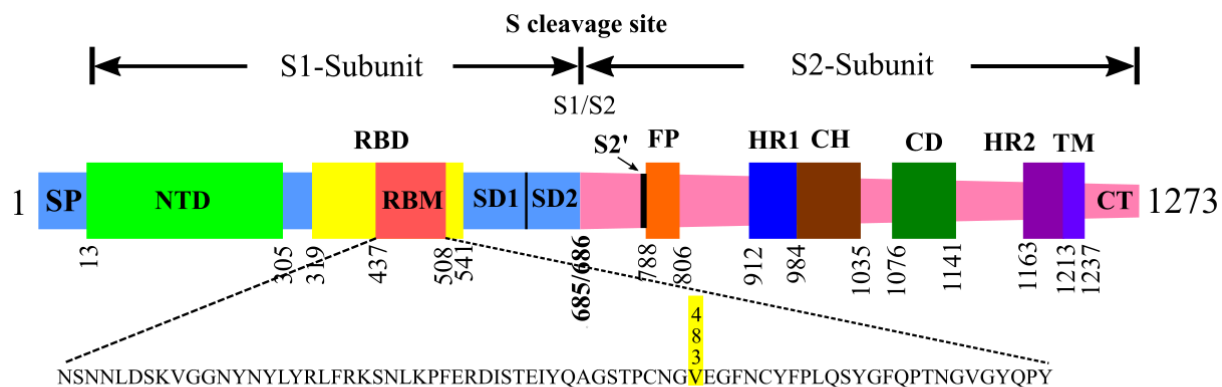
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Abstract | Exploring the biological significance of mutations in SARS-CoV-2 coronavirus, causing the COVID-19 pandemic, has recently become an area of paramount interest for many researchers, who are pouring their tremendous efforts, in cracking the COVID-19 pandemic code. One of many such mutations that have occurred in the viral genome is V483A mutation, which is a part of the receptor binding motif (RBM), present in the S1 domain of the spike protein. V483A mutant virus is becoming popular in North America with 36 cases so far, due to its frequent occurrences in the recent days. In this review, we have assembled all information, currently available on V483A mutation, and have made a critical analysis based on the perspectives of many researchers all around the world. Comparison is made between the wild type and the V483A mutants to analyze certain factors like type of interaction between the virus and host cell interface, binding affinity, stability, partition energy, hydrophobicity, occurrence rate, and transmissibility. *In silico* dynamic analysis shows minimal alteration in the receptor binding domain (RBD) of V483A mutant protein in free-state and no significant change of mutant tertiary structure of RBM upon binding to the ACE2 receptor. Comprehensive details about infectivity and evasion of the immune system by the virus are discussed. This information can in turn be of monumental importance in the field of vaccine and drug development because the mutants are becoming resistant to the vaccines and monoclonal antibodies.

Keywords | Spike protein; V483A; substitution mutation; virus-host cell interaction; high transmission; infectivity

Introduction

The human society is presently witnessing a new lockdown world that has emerged out of the progressing COVID-19 crisis. It seems as if the virulent nature of the SARS-CoV-2 has increased over the past few months, and its infection is continuously sweeping the world at a merciless infection rate. Researchers and scientists all around the globe are scrambling their resources and struggling to find a suitable solution to curtail the further spread of this deadly virus. The viral architecture of the SARS-CoV-2 contains four different structural proteins, namely Nucleocapsid (N), Membrane (M), Envelope (E) and Spike (S) protein.¹ The S protein consists of two subunits S1 and S2, with a furin cleavage site S'. The S1 domain of the S protein contains the receptor-binding domain (RBD), and the S2 domain has the fusion peptide domain. The spike protein of the virus contains ~1273 amino acids, where the S1 domain starts from 13 to 685 amino acid, and the S2 domain is from 686 to 1273 amino acid (FIG. 1).² The RBD of S1 binds to the human ACE2 receptor which is present on the epithelial cells. The fusion peptides present in the S2 subunit fuses to the ACE2 receptor and enables a successful entry into the host cell. The presence of furin cleavage site at the S protein differentiates it from other beta corona viruses. The ubiquitous expression of proteases in the host cell cleaves the S protein and aids in structural rearrangement and then assist in the successful fusion of the virus with the host cell thus infiltrating the host cell.³



SP - Signal peptide

NTD - N Terminal domain

RBD - Receptor binding domain

RBM - Receptor binding motif

SD1 - Subdomain 1

SD2 - Subdomain 2

S1/S2 - Protease cleavage site

S2' - Protease cleavage site

FP - Fusion peptide

HR1 - Heptad region 1

CH - Central helix

CD - Connector domain

HR2 - Heptad region 2

TM - Transmembrane domain

CT - Cytoplasmic tail

Fig. 1 | Schematic representation of the domains in the S protein of SARS-CoV-2.

Mutations in SARS-CoV-2 are causing another area of concern during the pandemic. It is found that the SARS-CoV-2 is mutating more rapidly, and till date, over 82062 mutations has occurred and it is still counting.⁴ The intervention of the human immune system is one of the reasons for such rapid mutation in the viral genome. Due to such rapid mutation, there is high variability in the genome and thereby posing a challenge for the scientists to find a suitable drug or vaccine. It is feared that the mutations can bring sequence variation and structural changes in the virus which can lower the effect of vaccines on these viruses by evading the host immune system through antibody escape.⁵ During this outbreak, many ongoing studies are focused on vaccine designing, drug repurposing, understanding the pathogenicity of virus, etc., the study of single amino acid mutation analysis is done very extensive. Mutation study on S protein helps in understanding the virulence of the virus, its antibody escape variants as well as its cellular tropism. Especially mutation study of the residues at the interface of the RBM and ACE2 has a significant role in potential pharmacophores for developing therapeutic drugs.⁵ In recent studies, the S protein has been looked upon as a potential immunogen because it is seen as the most accessible part of the virus and this protein is majorly responsible for the high infection rate of the virus.⁶ Among many other mutations, the mutations at the RBM are seen as hot spot which can potentially lead to higher infection rate.

In this review, we have singled out one such mutation, the V483A. It is an important amino acid residue in the RBM region of the spike glycoprotein, where the Valine at position 483 has changed to Alanine (FIG. 2), making the viral genome a unique mutant strain.⁷ V483A is one of the few mutations that has the potential to change the protein secondary structure and relative solvent accessibility in the RBM region.⁸ The RBM makes the contacts between the SARS-CoV-2 and the human ACE2 receptor acting as the core binding site. The schematic representation of domains and motifs, as well as the V483 amino acid residue position in the SARS-CoV-2 spike protein, are shown in the FIG. 1.

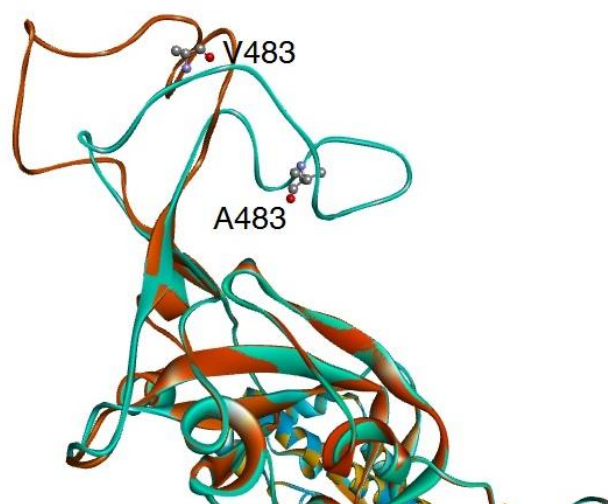


Fig. 2 | Loop configuration-reference structure is in orange and mutant is in blue.

Furthermore, the RNA replication rate causes the virus to mutate at a faster rate evading host immunity, thereby posing strong drug resistance. This mutagenic capability of the virus has become the leading cause of its evolution and genomic variation.⁹ This review aims at comprising all currently available information of the V483A mutant type and its characteristics in comparison with the wild type strain.

Transition of nucleotide induces the substitution mutation

Due to the transition from Thymine (Uracil) to Cytosine at the genome position 23010, the new clade or sub strain of the virus has evolved. Thus, Valine at the 483rd position of S protein got substituted to Alanine.¹⁰ This kind of nonsynonymous (i.e., amino acid altering) substitution mutation is getting increased in this novel Corona Virus. The nonsynonymous functional mutation in retroviruses will bring functional constraints to the protein and the mutation is a part of natural selection.¹¹ Many ssRNA viruses show stringent (Darwinian) selection to bring about positive changes in the virus which favors its transmission.¹² The nonsynonymous mutation is increasing at a higher rate in the RBD of spike protein.¹³ V483A mutation brings not only the primary and secondary changes to the viral S protein but helps to attain additional advantages like higher solvent accessibility, facilitates tighter binding to the receptor and in antibody escape. This mutation can be one of the favorable mutations of the virus to increase its transmission rate.¹⁴

Structure analysis of mutant protein

In V483A mutation, the amino acid Valine, which is hydrophobic in nature, present at the 483rd position of the Spike protein at the Receptor Binding Domain (RBD) of S1 got substituted as hydrophobic Alanine in some of the sequences. This mutation is present in the loop region of the RBD of S1.¹¹ This mutation is reported as one of the frequent mutations. The mutation occurrence from the month of March, 2020 till date is reported as 36⁶ (FIG. 3) and thus this mutant is found to be more prevalent in the United States.¹⁵



Fig. 3 | The wild strain from China- Wuhan got mutated and transmitted to various parts of the world as V483A variant.

The overall secondary structure upon binding to the ACE2 receptor for both reference and the mutant did not change hugely (FIG. 4). But upon binding there is a change in the RBD loop region where an increase of 1% is observed in the bend, structure (Helix and Beta sheet) and A- helix of mutant protein.¹⁴ The V483A mutation site does not form direct contact with the ACE2 receptor, but it is on the same face of the RBD that forms the binding interface with the ACE2.¹⁶ Along with V483A other mutations were reported at the 483rd position of the RBD where Valine is getting substituted to other amino acids such as Phenylalanine (F), Isoleucine (I), Proline (P), Aspartic acid (D), Arginine (R), and Lysine (K) in very low occurrence rate.^{17,18}

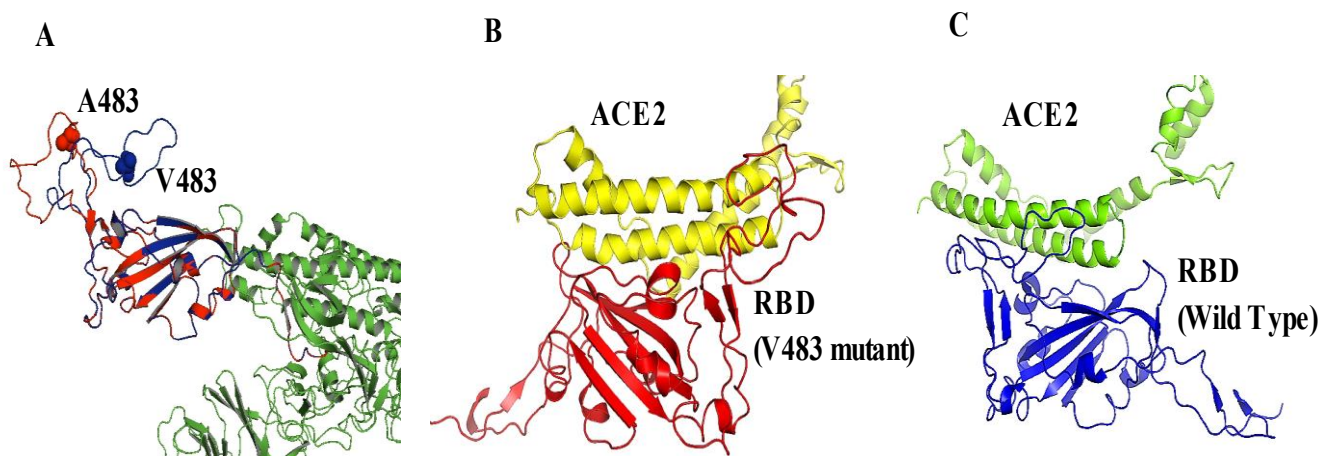


Fig. 4 | Spike protein of SARS-CoV-2. A) The RBD of S1 domain of the wild type is in blue and mutant in red, B) The docked pose of mutant V483A RBD interacting with human ACE2 receptor helix1, C) The docked pose of reference RBD to the human ACE2 receptor helix1.¹¹

Binding energy comparison

The free binding energy (ΔG) of the reference type S protein to the human ACE2 receptor is -14.1 Kcal/mol and for the V483A mutant it is -15.2 Kcal/mol.⁷ Therefore, the change in the binding energy after mutation is $\Delta\Delta G$, which is calculated by calculating the difference between the free binding energy ΔG of reference type from the mutant.¹⁹ For V483A the $\Delta\Delta G$ is found to be ($\Delta\Delta G = \Delta G$ wild type - ΔG mutant) +1.1 Kcal/mol. The $\Delta\Delta G$ value is positive, so the mutation increases the binding affinity and hence the mutant has the capacity to become more stable and more infective in future.¹⁹ The short-range Coulombic interaction energy between the RBD of reference and human ACE2 receptor in a dynamic environment is -2.307×10^5 Kcal/mol and for V483A mutant it is -2.320×10^5 Kcal/mol. Thus, the mutant protein can bind much better and can be stable during the interaction with the ACE2 receptor when compared to the wild type.²⁰ Hydrogen bonding (H-bonds) is an exchange reaction during mutations to form protein-protein complex. New hydrogen bonds are formed during the interaction to form the protein-protein complex. The number of H-bond may play an important role in stabilizing the two molecules.²¹ The number of H-bonds involved with wild type and the V483A mutant is estimated by 20 ns simulation using molecular dynamics (MD) studies. The average number of H-bonds between ACE2 receptor and V483A mutant is 7.283 ± 1.568 whereas with the reference S protein, it is found to be 5.651.²⁰ Therefore, the outcome predicts that the mutated model forms highly stable complex with ACE2 receptor rather than its wild type. The stability of protein-protein complex is also determined by the root mean square deviation (RMSD) analysis. The docking studies have been performed between RBD region of the spike protein and the ACE2 receptor. The RMSD values are on average 3.6 ± 0.57 Å and 3 ± 0.43 Å for reference and mutated residue at V483 respectively.²⁰ FIG. 5 represents the 3D model of the reference (wild type) and the mutant S protein RBD binding to the human ACE2 receptor along with the H-bond details in ball and stick representation. Also Table 1 details about the H-bond interaction between the S-protein and the receptor with the bond distance.²⁰

Table 1 The amino acids of S protein which are involved in interacting with the human ACE2 receptor-A chain of helix 1 in the wild type and mutant are tabulated below. ²⁰

H-bond interaction between hACE2 receptor and amino acid residues		Hydrophobic interaction between S protein and hACE2 receptor.	
Wild type (Reference)	Mutated (V483A)	Wild type (Reference)	Mutated (V483A)
Tyr41- Thr500 (2.68)	Tyr41- Thr500 (2.75)	Gly354	Met82
Gln24- Asn487 (2.63)	Gln24- Asn487 (2.45)	Tyr505	Tyr83
Lys31- Gln493 (2.97)	Lys31- Gln493 (2.58)	Asn501	Gly476
Lys31- Glu484 (2.84)	Lys31- Glu484 (2.40)	Met82	Phe486
Lys353- Gly502 (3.01)	Lys353- Gly502 (2.97)	Phe486	His34
Lys353- Gly496 (2.79)	Lys353- Gly496 (2.68)	Thr27	Leu455
Lys353- Gln498 (2.75)	Lys353- Gly498 (2.88)	Phe28	Thr27
Asn330- Thr500 (3.26)	Asn330- Thr500 (3.02)	Tyr473	Phe456
Gln42- Tyr449 (2.68)	Gln42- Gly446 (2.76)	Ala473	Tyr473
Gln42- Gly446 (2.84)	Gln42- Tyr449 (2.91)	Tyr489	Phe28
Asp38- Tyr449 (2.39)	Asp38- Tyr449 (2.44)	His34	Tyr489
Asp30- Lys47 (2.40)	Asp30- Lys417 (2.50)	Phe456	Gly354
Asp355- Thr500 (3.24)	Ser19- Ala475 (2.30)	Leu455	Gln493
Tyr83- Asn487 (3.15)	Glu37- Tyr505 (2.67)		Asn501
Glu35- Gln493 (2.94)			

Note: The amino acids of S Protein are in black and for the human ACE2 receptor in bold black letters

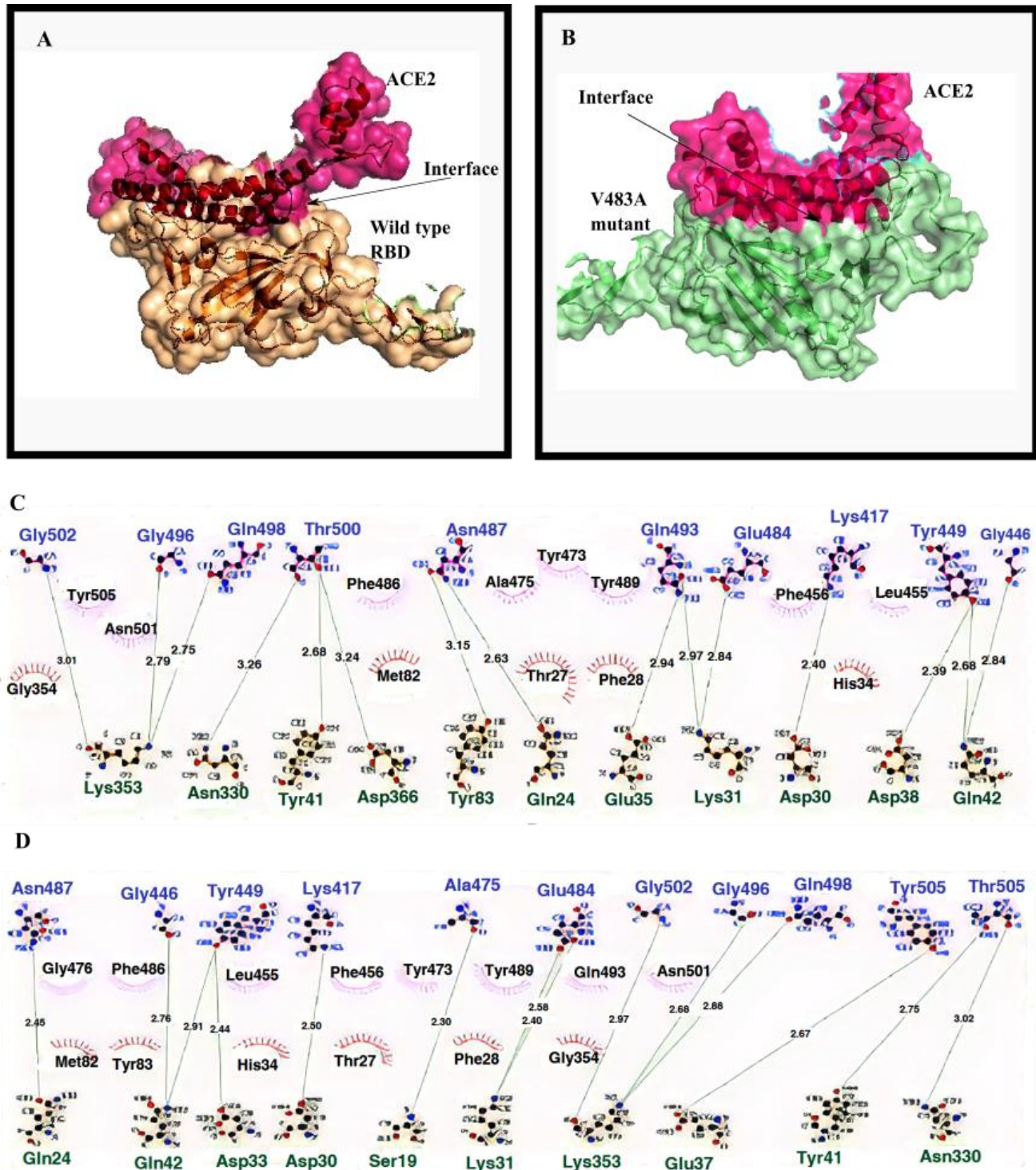


Fig. 5 | 3D representation of the interaction between the RBD of S1-Domain with the human ACE2 receptor helix 1- chain A Wildtype S-protein in (3.A) and mutant V483A in (3.B). 3- C and D represents the ball and stick model of interacting amino acids of wildtype and V483A mutant respectively where aa in blue belongs to S protein and green belongs to human ACE2 receptor.²⁰

Role of mutation in increasing the stability

Though the V483A mutation is at the receptor binding motif of the S1 protein, the amino acid is not making direct contact with the ACE2 receptor. When compared with SARS-CoV-1 this mutant protein is binding 4 to 10 fold tightly to the receptor.¹³ The V483A mutation with higher frequency indicates that this mutation may favor SARS-CoV-2 by natural selection and may cause this virus to be more infectious. Positive binding affinity also indicates that the mutation will help in making tighter interaction with the receptor.¹⁵ Cryo EM studies show that this type of nonsynonymous single nucleotide variations may affect the strength of transmission of the virus.²² The dynamic studies on a structural basis due to this mutation revealed that the binding surface of the RBD to ACE2 is having largely random coil conformation and it is lacking structural rigidity. To get the firm scaffold, beta-sheet structures are provided by the 510-524 amino acids of S protein.²³ The V483 mutant site in the RBD of S1 is in close proximity of Q24 of ACE2. V483 is more than 10 Å away from the Q24, one of the interacting amino acid of ACE2 and still it could affect the receptor binding of SARS-CoV-2 by indirectly altering the loop region of the RBM of S protein and hence leads to more stability.²⁴ The V483A mutant is exposed to solvents as they present on the surface of the RBD and the loop region may stick out into the solvent. Thus, this mutation may not directly impact the receptor binding or stability, but it lowers the hydrophobic surface and also lowers the non-specific stickiness of this loop region, and may affect the binding of antibody.²⁴ The Kyte-Doolittle hydrophobicity index value for the V483A mutant is -2.4, where the negative value indicates the hydrophilic nature of the loop region. It is known that Valine hydrophobic index (4.2) is higher than Alanine hydrophobic index (1.2).¹³ Results of dynamic study for around 300ns show that this variant is stable throughout the simulation.⁵ From an evolutionary perspective, this mutation may further evolve to be an even more dangerous sub-strain to humans.⁵ All the non-bonded interactions arising between the virus and the host cell receptor, it is observed that an elimination of a hydrogen bond with the receptors caused due to mutations would give rise to a 20% increase in co-ordination the number or in number of contacts between the virus and the host cell receptors.²⁵

Other amino acid substitution at V483

In case of V483F mutation the amino acid Valine, which is hydrophobic is substituted by bulky hydrophobic amino acid phenylalanine and this might influence the efficiency of glycosylation of nearby amino acid N343 or the positioning of sugars.²⁴ There are other mutations occurring at this hotspot where Valine is replaced by other amino acids such as Proline, Aspartic acid, Lysine with low occurrence rate. Their maximum binding energy difference ($\Delta\Delta G$) is 3.162 Kcal/mol and 0.05Kcal/mol

for Proline and Aspartic acid respectively. This positive binding energy indicates that these amino acid substitutions can also lead to tighter binding of S protein to the receptor. For Lysine the minimum $\Delta\Delta G$ is reported to be -0.851 Kcal/mol.¹⁸ Thus, these mutations can also have the potential to emerge as one of the infectious strains (Table 2).

Table 2 Overall summary of all the mutations at the 483rd position of V483 of RBM in S1 domain.

SNP position	Nucleotide base substitution	Amino acid substitution	No. of occurrences	No. of variants	Clade
23010	T to C (Transition)	V483A	36 ⁶	A,F,I,P,D,R,K ^{17,18}	S84 ²⁶

Immune evasion by mutant

Neutralizing antibodies evoked by either natural infection or vaccination are the beginning of building adaptive immunity of the populace against SARS-CoV-2.²⁷ Passively administering antibodies as a recombinant protein or convalescent plasma is one of the effective therapeutic and prophylactic measures that can be taken against its infection.²⁸ The emergence of antibody-resistant variants of this virus hindering the therapy can be brought under control by combining antibodies towards neutralizing epitopes.²⁹

Studies show that the neutralizing antibody response to infection is critical for creating an effective and durable vaccine.³⁰ Investigations on the infectivity and reactivity of the V483A variants showed that they were resistant to some neutralizing antibodies. These findings could be of value in the development of vaccine and therapeutic antibodies.⁶ A change in the amino acid residue of the RBM of the spike protein can give rise to significant changes in the functionality, infectivity, transmission as well as the interactions of the virus with neutralizing antibodies.³¹ The neutralizing antibodies are those which bind to the virus and neutralize its effect on the host system.³² Analysis on the antigenicity of V483A mutant using monoclonal antibodies (mAbs) revealed that V483A became resistant to X593 and P2B-2F6 mAbs. These two monoclonal neutralizing antibody works better for reference strain, but its activity is 10 fold decreased sensitivity in the mutant strain when compared with reference strain.⁶

Antibody studies conducted using the antibody 5A6 immunoglobulin-G which has the superior neutralization capacity with many SARS-CoV-2 mutant strains, including the D614G strain, failed to neutralize the V483A mutant strain. Although the antibody 5A6 IgG had a high occupancy on the viral surface and had bivalent binding capacity binding to both the “up” and “down” positions of the RBD-ACE2 interaction surface, showed a 4-fold reduction in binding avidity to the V483A mutant strain and so it is recommended to administer the antibody 3D11 along with 5A6 IgG to compensate this failure against the V483A strain.³³

Scope of vaccine design

Epitope analysis of the V483A mutant strain proved that there are 13 effective B-Cell epitopes that significantly advanced the antigenicity of the mutant as compared to the wild type strain they were 62~75, 487~492, 210~221, 181~186,342~353,363~377, 617~628, 405~418, 405~413, 379~389, 442~447, 458~463, and 698~709. Although this epitopes account for very small proportion of the population, precautions must be taken against any kind of antigen escape induced by genetic variation during vaccination.³⁴ One of the *insilico* study using NetMHC4 software binding affinity between the epitope of S protein to class I major histocompatibility (MHC) with using the most frequent HLA is predicted and the software predicted 9-mer (eg., GAEGFNCYF epitope) in which the MHC I can bind effectively to the mutant strain. Because of positive affinity and varied solvent accessibility it will create negative impacts on the vaccine and diagnostic test development. In a recent study the multi epitope vaccine designed using DeepVacPred software shows effectively binding for mutant strain also.³⁵

Although carefully selected therapeutic cocktails will offer greater resistance to SARS-CoV-2 escape, these identified mAbs will greatly help in the preclinical evaluation and development of immune therapeutics to be used against COVID-19 in humans.³⁶ Since mutations bring about changes only in the spike protein structure without any differences in the ACE2 receptor moiety, it is predicted that vaccines developed to bring around the immunogenicity of humans in fighting the virus cannot be affected by mutations, except if there are any aggressive mutations. The V483A has not been reported till date, as an aggressive mutation, although it is one of the most important mutations after the D614G mutation.¹⁹ In any case, exploring the complete nature of the virus along with each of its mutant strains has always been of paramount importance in designing an effective vaccine and for meaningful therapeutic treatments.⁵ A list of protein-based vaccines that are currently undergoing clinical trial has been given in the table below (Table 3).

Table 3 List of vaccines under clinical trials for vaccine development of COVID-19. ³⁷

Vaccine candidate	Vaccine platform	Developer/Manufacturer/Country	Expected outcomes/Results
ChAdOx1-S (AZD1222)	Non-replicating viral vector	University of Oxford/AstraZeneca/UK	Enhance the immune response against the spike protein of SARS-CoV-2, which will able to restrict the entry of virus to human cell and can prevent the infection.
LNP-encapsulated mRNA (mRNA-1273)	RNA	Moderna/NIAID/USA	Block spike protein binding ability with ACE2, as well stopped its consequences and proliferation.
Adenovirus Type 5 Vector (Ad5-nCoV)	Non-Replicating Viral Vector	CanSino Biological Inc./Beijing Institute of Biotechnology/China	It can neutralize RBD-specific ELISA antibody response to control the deadly virus.
Adjuvanted recombinant protein (RBD-Dimer)	Protein Subunit	Anhui Zhifei Longcom Biopharmaceutical/Institute of Microbiology, Chinese Academy of Sciences/China	The RBD is important for immune response, therefore it is an attractive target vaccine and RBD-Dimer restricts to bind with receptors and control its interference.
DNA plasmid vaccine with electroporation (INO-4800)	DNA	Inovio Pharmaceuticals/International Vaccine Institute/South Korea	It is able to block the spike protein and ACE2 receptor of host cell by neutralizing SARS-CoV-2 infection, as well as has the capability to work against mutant variant, D614.
Ad26COVS1	Non-Replicating Viral Vector	Janssen Pharmaceutical Companies/USA and Belgium	Effectively neutralize the antibody and enhanced immunity response against SARS-CoV-2 glycoprotein and can stop interaction between glycoprotein and ACE2 receptor of host cell.
RBD-based	Protein Subunit	Kentucky Bioprocessing, Inc/USA	Initiate antibodies to prevent binding of subunit (S1/S2) with receptor and later regulate the membrane fusion to restrain the virus infection.
Native like Trimeric subunit Spike Protein vaccine (SCB-2019)	Protein Subunit	Clover Biopharmaceuticals Inc./GSK/Dynavax/Australia	
Recombinant spike protein with Advax™ adjuvant	Protein Subunit	Vaxine Pty Ltd/Medytox/Australia	
Molecular clamp stabilized Spike protein with MF59 adjuvant	Protein Subunit	University of Queensland/CSL/Seqirus/Australia	
S-2P protein + CpG 1018	Protein Subunit	Medigen Vaccine Biologics Corporation/NIAID/Dynavax/USA	
Full length recombinant SARS CoV-2 glycoprotein nanoparticle vaccine adjuvanted with Matrix M	Protein Subunit	Novavax/USA	Highly immunogenic response with specific antibodies that can deactivate binding capability of spike protein of virus with receptor present in human cell, as well neutralize the antibodies of SARS-CoV-2 wild-type virus and restrict its domain activity.

Conclusions

The V483A mutation represents one of the major emerging mutations of the current COVID-19 pandemic crisis. We found that significant attention has been given by various researchers globally to this particular variant. Data from high-quality research works were coordinated, and critically reviewed in all sections of this review. Evidences coming from different researchers worldwide show that it is the next emerging mutation after D614G that can severely enhance the infection rate. V483A is not directly related with the virus-host cell interaction, but it has the ability to enhance the binding stability and binding capacity of the protein-protein complex. We observed that the V483A mutation was first observed in the North American region and its occurrence is now predominantly increasing in its population, as well spreading towards European and Asian region. It is also assumed that this mutation can be one of the key factors for the higher death rate in the USA. Furthermore, we have highlighted all possible angles and evidences that can help researchers to get a clear picture of this SARS-CoV-2 variant, and to investigate potential therapies for its neutralization. We believe the current circumstances justify prioritization of such mutation studies, and there is sufficient insight and a rationale that needs to be suggested to scientists, on whom the world depends for the inception of a vaccine, that can spell the end of this deadly virus.

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Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

The authors declare no competing interests.